TECHNICAL PAPERS

Chemotherapeutic Investigations of Cyanine Dyes

L. G. S. BROOKER

Research Laboratories, Eastman Kodak Company, Rochester, New York

L. A. SWEET

Research Laboratories, Parke, Davis & Company, Detroit, Michigan

Although extensive investigations have been carried out on the chemotherapeutic properties of the cyanine, styryl, and related dyes by Prof. Browning and his collaborators at the University of Glasgow (*1*), the chief use of these dyes has remained in the field of photography, where they are indispensable as color sensitizers. Because of their significance in this connection, a very large number of new dyes of these classes has been prepared during the past 20 years in the Research Laboratories of the Eastman Kodak Company, in the department of one of us (L. B.) who, prompted by the earlier work of Browning, was eager to see the pharmacological investigations extended and the newer cyanine dye types in particular given adequate chemotherapeutic testing. An arrangement was reached whereby certain representative compounds were prepared[1] in a suitably soluble form and submitted for extensive testing.

Some of the dyes were supplied to the National Research Council Chemotherapy Center for Tropical Diseases for testing against the organisms of such diseases. The most striking fact to emerge from the investigations which ensued was the pronounced antifilarial and anthelmintic activities of many of the cyanine types.[2] Many of the dyes also showed antimalarial activity, but not such as to equal that of drugs already available. The antimalarial results have been reported (*2*).

Another extremely interesting activity was noted in our laboratories when (1-amyl-2,5-dimethyl-3-pyrrole) (1,6-dimethyl-2-quinoline) dimethinecyanine chloride was tested *in vitro* against the lactic acid-producing bacilli.[3] This cyanine dye completely inhibited the growth of *Lactobacillus casei* at 2×10^{-6} M. *L. arabinosus* was inhibited by a concentration of 2×10^{-6} M, *Streptococcus faecalis* by 4×10^{-6} M, and *Escherichia coli* by 3×10^{-6} M. The inhibition of *E. coli* by 3×10^{-6} M concentration of cyanine dye was partially reversed by 100 mg./100 cc. of vitamins B_1 and B_2, nicotinic acid, and pantothenic acid. The reversal by crude yeast and liver extracts was even greater than indicated by their vitamin content. Vitamin B_6 and p-aminobenzoic acid were without effect on the inhibitory action of the dye.

In order to determine the nature of the bacterial inhibition, the effect of the cyanines on various enzyme systems was studied. There was no appreciable effect on the d-amino acid oxidase, cytochrome oxidase-cytochrome C, succinic dehydrogenase, glucose dehydrogenase, lactic dehydrogenase, and liver glyoxalase enzyme systems.

Representative dyes were also tested *in vivo* for activity against *Str. hemolyticus, Str. viridans, Staph. aureus, Diplococcus pneumoniae I, Trypanosoma equiperdum, Treponema pallidum*, St. Louis encephalitis, influenza, typhus, and other viruses, and a group of intestinal parasites; also *in vitro* against *Endamoeba histolytica*.[3] While an occasional compound showed some demonstrable activity against these organisms, the order of activity was not sufficiently great to be therapeutically significant.

A summary of the cyanine, styryl, and related dyes studied will be supplied on request.

References

1. BROWNING, C. H. *Edinb. med. J.*, 1937, N.S. 44, 497.
2. WISELOGLE, F. Y. (Ed.) *A survey of antimalarial drugs*. Ann Arbor, Mich.: Edwards Bros., 1947.

[1] The dye syntheses were carried out by E. Van Lare, R. H. Sprague, F. L. White, G. H. Keyes, and Miss G. VanZandt, to whom the authors acknowledge their indebtedness.

[2] These investigations will be reported at the May meeting of the Federated Biological Societies in Chicago by R. N. Bieter and A. D. Welch and their associates.

[3] The authors acknowledge the work of F. D. Stimpert, O. M. Gruhzit, O. D. Bird, and G. Rodney, of the Research Laboratories, Parke, Davis & Company, under whose supervision many of these tests were performed.

Hereditary Obesity and Efficient Food Utilization in Mice[1]

G. E. DICKERSON

Regional Swine Breeding Laboratory, Ames, Iowa

J. W. GOWEN

Iowa State College, Ames, Iowa

Energy costs of transferring food materials into body tissues constitute a recurrent problem of genetics, nutrition, and physiology. The specific problem of inherited fatness in relation to efficiency of food utilization came to our immediate attention in a study of rate and economy of gain and carcass composition of swine (*3*). It was found that the hereditary influences which reduced the food required per pound of gain also increased the proportion of the gain that was fatty tissue. These results were at variance with the supposition that food requirements would be larger for deposition of fatty tissue, because of its higher energy content, compared with nonfatty tissue. This supposition apparently holds for the

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increase in fat deposition during the later stages of development of a given animal. However, we were concerned with the effect of hereditary differences in the composition of weight gains on food required per unit of gain among different animals at a similar stage of growth. This may involve heritable differences in food consumption and in energy expended for activity and other body work, as well as differences between fat, carbohydrate (*5*), and protein in the energy required for transfer from food to body tissue.

Because of certain limitations of the swine data and the broad implications of the findings, it became desirable to study more critically the nature of inherited fatness in relation to food utilization. The marked adiposity induced in mice by the "yellow" gene (*2*) seemed to offer unusual opportunity for the controlled experiment (*4*) which is reported briefly here.

Because yellow coat color in the mouse is produced by a dominant autosomal gene in the heterozygous condition, crosses to nonyellow mice give approximately equal numbers of yellow and nonyellow progeny within each sex. Accordingly, yellow and black agouti segregates were obtained by crossing yellow males of a highly inbred stock, kindly provided by L. C. Dunn, with albino females of another highly inbred

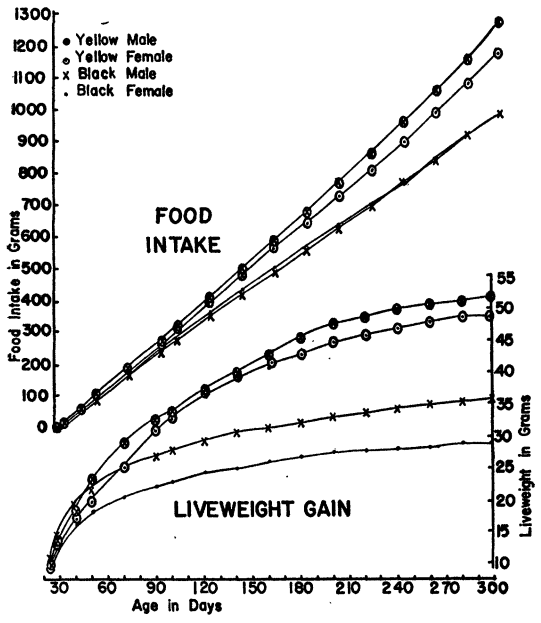

FIG. 1

line of our own. The yellow and black littermates of the same sex differed presumably only in the one chromosome or portion thereof which carried the "yellow" gene. Sets of unmated yellow and black littermates were compared within each sex and growth period, the periods being from 25 days to 30, 50, 90, 200, and 300 days of age. Each such series of comparisons was replicated four times, at approximately monthly intervals, to measure seasonal and litter variation in the results. A total of 62 yellow and 65 black mice were used. The mice were self-fed in individual cages to permit full expression of genetic differences in appetite as well as in utilization of food consumed. The only food was a complete, finely ground ration fed in shallow jelly glasses with access through a hole in the cover. The shelves containing the feeding cages were completely enclosed by fine screen to prevent food loss to stray mice. Feces, urine, and spilled food were recovered on waterproofed paper trays. Live weight gains, food consumption, and feces production were obtained by 10-day periods from 30 to 50 days and by 20-day periods thereafter. At the close of its feeding period each mouse was chloroformed, and its whole body was analyzed for fat, nitrogen, water, and total dry matter by Prof. Wilkinson, of our Experiment Station chemistry staff. Body composition was also obtained for yellow and black mice from two litters within each sex at 25 days of age.

The curves in Fig. 1 show the average gain and food consumption for all sets of yellow and black littermates within each sex and age. From 25 to 35 or 40 days of age, yellow and black mice of the same sex were nearly alike in both gain and food consumption, and the gain was predominantly protein. After 40 days, yellow mice of both sexes exceeded their black littermates greatly in gains but only moderately in food consumption. It is evident from Fig. 2 that the extra gain of the yellow mice was entirely fat tissue. Hence, it is immediately clear that the "yellow" gene greatly increases the energy stored per gram of gain but at the same time sharply reduces the food required per gram (and even more so per calorie) of gain. For example, during the period from

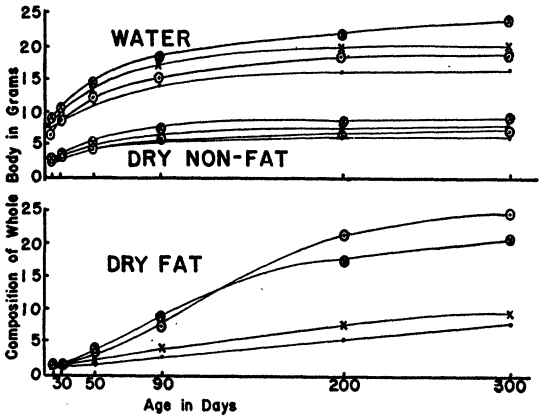

FIG. 2

40 to 90 days of age, food requirements per gram of gain were only .44 as much for yellow as for black females and .66 as much for yellow as for black males. The greater efficiency of the yellow mice is even more pronounced when the comparison is made from weaning to a fixed final weight, so that the faster gain of the yellows directly reduces the number of days fed. For example, from 25 days to a final weight of 25 grams in females and 30 grams in males, the ratios of food consumed per gram of gain by yellow to that by black mice were .38 in females and .51 in males.

The yellow gene accomplished the increased fat deposition and the lowered food requirement per unit of gain by increasing the appetite slightly and reducing the energy expended in body work, especially in activity, beginning at 35 or 40 days of age. Rytand (*9*) also observed this increased appetite of

yellow mice. Judging by the weights and average composition of feces, the percentage of the food calories eliminated in the feces (and in the urine absorbed by the feces) ranged from about 19 in the 25–30-day period to about 23 in the 25–300-day period. It was slightly lower for black females than for the other three groups, perhaps because more urine was absorbed by spilled food and less by feces for the former. Thus, the increase in nutrients absorbed by yellow, as compared with black, mice was nearly in proportion to the increase in food consumption. Furthermore, the increase in energy expended in body work by the yellow mice was proportionally much less than the increase in their average body weight, particularly in females. For example, the increase of yellow over black mice in calories absorbed from 25 to 300 days of age was 20 per cent in females and 34 per cent in males, whereas the increase in calories stored in the body was 210 and 114 per cent, respectively. Energy used for body work (food less feces and gains) increased only 15 per cent in females and 32 per cent in males compared with increases of 56 and 39 per cent, respectively, in average body weight for the period. Taking calculated energy expended in body work per unit of average body weight as 1.00 for black females, relative values for black males and for yellow females and males were .82, .74, and .78, respectively. Similar results were obtained during periods when mature mice (usually over 180 days of age) made little or no change in weight. Here it was found that food requirements per gram of body weight were only .75 as large for yellow females and males and .87 as large for black males as those for black females. These results corresponded with our observation throughout the experiment, that black females were much more excitable and active than yellow females or males, with black males intermediate. The amount of food thrown from the dishes provided a crude index of activity. Limited observations on mature mice indicated that daily food spillage per gram of body weight was 1.3 grams for black females but only .20, .05, and .17 gram, respectively, for black males and for yellow females and males.

The data emphasize how little of the food energy is stored in the body compared with that used for the maintenance and activity of the body. Energy stored as fat and protein represented only 2–11 per cent of the total food energy during the several periods from 25 to 300 days of age, whereas energy expended in body work represented 70–80 per cent. Of the total calories consumed, the yellow mice stored 2–5 per cent more than their black littermates and used at least that much less for body maintenance and activity. A relatively small reduction in maintenance food thus causes a large increase in food stored.

The work of Miss Weitze (*11*) has only recently come to our attention. Her results agree closely with ours with respect to the effect of the "yellow" gene on the rate and composition of gain and on appetite. However, she found no effect on activity as measured by a treadmill or on food energy used for maintenance and activity per unit of body weight. These discrepancies may be due to inadequacy of a treadmill for measuring natural activity and to the fact that metabolism data was obtained for only one yellow mouse of each sex, using average figures for composition of the gains. Her results from parabiosis, hypophysectomy, and histological studies indicate that the action of the "yellow" gene is hormonal, involving altered carbohydrate metabolism.

There is evidence that the action of the "yellow" gene is similar to that of the genes affecting fat deposition in animals generally. Recent correspondence with Miss Kennedy indicates that the strain of rats produced at the Minnesota station (*7*) by selecting for efficient food utilization gained more rapidly and required about 30 per cent less food per gram of gain and unit of body weight than the strain selected for inefficient gains, under full feeding. The extra gain of the "efficient" strain was largely fat tissue. Further evidence is provided by MacArthur's (*6*) selection for slow and for rapid growth to 60 days of age in two strains of mice. After 8 generations, mice of one strain were twice as large as those of the other, and mice of the large strain "tend to become sluggish and obese," whereas mice of the small strain "are very active and aggressive," suggesting a difference in metabolic rate. Differences in activity rather than in resting metabolism are indicated by Benedict's (*1*) finding that the heat production of resting mature mice per unit of calculated surface area ($\frac{2}{3}$ power of weight) was the same for an exceedingly fat race (MacDowell's CHS Silvers, not "yellows") as for common laboratory and wild mice. Salcedo (*10*) found that mice of this same fat race oxidized tissue fat more slowly than normal mice when starved and interpreted this slower fat oxidation by the fat race as the cause of their obesity. However, both could be the result of inherently smaller energy requirements. Ritzman and Colovos (*8*) have shown how strikingly the genetic association of fatness with efficient gains is exemplified in the pig, as contrasted with sheep and cattle, because of its greater appetite and reduced heat losses and activity.

The evidence presented indicates that the "yellow" gene in mice reduces food requirements per unit of gain and produces obesity, primarily by increasing the food intake and by reducing energy expended, especially for activity. Both of these effects increase the energy available for storage as fat, whereas the first raises only slightly, and the second reduces, the energy dissipated in body work. Hence, the "yellow" gene causes gain in weight to increase much more than total food consumption, even though the extra gain is fat tissue containing more energy per unit of weight than other tissues. These results emphasize the distinction between the hereditary association of increased fat deposition with lower food requirements per unit gain in weight and the developmental association of increased fat deposition with higher food requirements. Hereditary obesity is the result of more efficient food utilization.

References

1. Benedict, F. G., and Lee, R. C. *Ann. Physiol.*, 1936, **12**, 1050.
2. Danforth, C. H. *Anat. Rec.*, 1925, **29**, 354; *Proc. Soc. exp. Biol. Med.*, 1926, **24**, 69; *J. Hered.*, 1927, **18**, 153.
3. Dickerson, G. E. *J. Animal Sci.*, 1943, **2**, 357.
4. Dickerson, G. E., and Gowen, J. W. *Rec. genet. Soc. Amer.*, 1946, **14**, 44.
5. Forbes, E. B., Swift, R. E., Elliot, R. F., and James, W. H. *J. Nutrition*, 1946, **31**, 203, 213.
6. MacArthur, J. W. *Amer. Nat.*, 1944, **78**, 142, 224.
7. Morris, H. P., Palmer, L. S., and Kennedy, Cornelia. *Minn. agric. Exp. Sta. tech. for consistency Bull. No. 92*, 1933.
8. Ritzman, E. G., and Colovos, N. F. *N. H. agric. Exp. Sta. tech. Bull. No. 75*, 1941.
9. Rytand, D. A. *Proc. Soc. exp. Biol. Med.*, 1943, **54**, 340.
10. Salcedo, Juan, Jr., and DeWitte, Stetten, Jr. *J. biol. Chem.*, 1943, **151**, 413.
11. Weitze, Maria. *Hereditary adiposity in mice and the cause of this anomaly*. København: Store Nordiske Videnskabsboghandel, 1940. (Ph.D. thesis.)